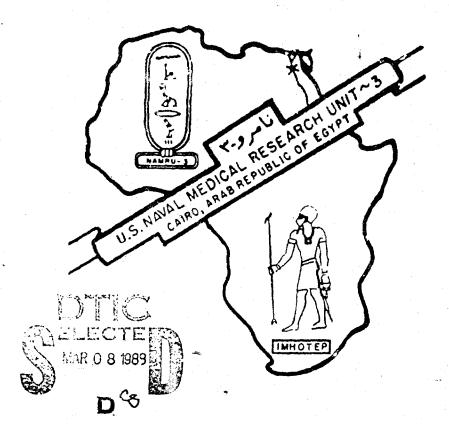
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NEUROSECRETORY CELL TYPES AND DISTRIBUTION IN UNFED FEMALE HYALOMMA DROMEDARIA (ACARI: IXODOIDEA: IXODIDAE) SYNGANGLION

BY

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Ву

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- * From Research Project 3M161102BSIO.AD.424; Naval Medical Research and Development Command, Naval Medical Command, National Capital Region, Bethesda, Maryland. Supported in part by contract RR041-26-01, NR205-040 with the Office of Naval Research, Microbiology Program, Navai Biology Project, Arlington, Virginia. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy or of the naval service at large.
- + Dedicated to late Dr. Harry Hoogstraal who initiated and supported this work before he passed away on 24 February **1986**.

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Neurosecretary Cell

Thirteen (NSC) types have been observed in the unfed female Hyalomma dromedarii synganglion. Distribution of these types was based on cell shape, size, and staining reaction of their contents. The NSC are grouped in 13 centers, namely protocerebral, cheliceral, stomodeal pons, palpal, oesophageal, globular, olfactory glomerular, four pedal, opisthosomal and postoesophageal. Each center contains one or more cell types.

INTRODUCTION

Gabé (1955) was the first to describe secretory activity within nerve cells in the synganglion of the argasid ticks Ornithodoros (Pavlovskyella) erraticus Lucas and O. (Alveonasus) lahorensis Neumann. Subsequently, neurosecretory cells (NSC) have been described in the ixodids Boophilus (Uroboophilus) microplus (Canestrini) (Binnington and Tatchell, 1973), Dermacentor (D.) pictus Herm (Ioffe, 1964), D. (D.) variabilis Say (Obenchain, 1974a, b; Obenchain and Oliver, 1975), Hyalomma (H.) asiaticum Schulze and Schlottke Ixodes (I.) ricinus (Linnaeus) (Ioffe, 1964), I. (I.) persulcatus Schulze (Panfilova, 1980), Rhipicephalus (R.) sanguineus (Latreille) (Chow and Wang, 1974), and the argasids Argas (Persicargas) persicus Oken (Eisen et al., 1973), O. (O.) savignyi (Audouin) (Evans and Solomon, 1977) and O. (P.) parkeri Cooley (Pound and Oliver, 1982).

The first attempt to classify the NSC into distinctive types was in O. (O.) moubata Murray (Eichenbergr, 1970). Later, Obenchain (1947b) presented an arbitrary classification scheme for the NSC in D. variabilis and Gabbay and Warburg (1977) in O. (P.) tholozani.

In H. (H.) dromedarii Koch, changes in the neurosecretory system have been correlated briefly with moulting and oviposition (Dhanda, 1967). In this study, we describe in detail the NSC types and their distribution in the synganglion of unfed female H. dromedarii to provide background data essential for

subsequent studies on changes in these cells associated with feeding and mating.

MATERIAL AND METHODS

Adult H. dromedarii were colonized in the NAMRU-3 Medical Zoology laboratory from females collected from camels in the Imbaba market, Giza, Egypt, using domestic rabbits as hosts. Methods of feeding and rearing were those of Berger et al. (1971) at $28 \pm 1^{\circ}\text{C}$ and 75% relative humidity. All animal care and manipulations were in accordance with the Animal Welfare Amendment of 1976 (PLP14-279) with subsequent amendments.

For histological examination, ticks were dissected on the day of moulting in a 0.7% saline solution and the synganglia were fixed in Bouin's fluid, dehydrated and double-embedded in celloidin-paraplast. Five to 7 μ -thick serial sections were stained using Mallory triple stain (MT) (Pantin, 1959), Ewen's (1962) paraldehyde-fuchsin (PF) and Gomori's (1941) chrome-haematoxylin-phloxine (CHP).

Cellular diameter of 6-10 specimens were measured. The surface area of sections of oblong cells

was calculated using the formula $A = \pi \times \frac{1}{4}$ where A is the

area, a is the small diameter and b is the large diameter. The mean and standard error were then calculated for each cell type and the data were compared using the Student's t-test.

RESULTS

Thirteen NSC types (I-XIII) were observed in different locations in the synganglion. Distinction of these types was based on cell shape, size and staining reaction of their contents as illustrated in Table 1.

Type I: These NSC are the largest of all cell types (P < 0.001). The monoaxonal cell is oval, and contains several peri-

pheral vacuoles and a large central nucleus with 3 nucleoli (Fig. 1). Some of the densely-distributed neurosecretory granules (NSG) clump together forming larger aggregates around the nucleus and others are traced in the axon.

Type II: These NSC are oval with a central nucleus having 2 nucleoli. Two subtypes are observed according to cell size and NSG amount and distribution. Subtype IIa cells have very fine granules enclosed in areas of clear, unstained cytoplasm which gives the cytoplasm a vacuolated appearance (Fig. 1). Smaller subtype IIb cells (P < 0.02) contain coarse granules with large accumulates on one side of the nucleus (Figs. 1, 4).

Type III: These NSC are large, cval and their nuclei contain 2 nucleoli. Two subtypes are distinguished according to cell size and NSG shape and distribution. In the larger subtype IIIa cells (P < 0.01) the cytoplasm contains several large vacuoles and the coarse NSG aggregate more densely at one side of the nucleus (Fig. 1). The subtype IIIb contains clumps of flocculant granules uniformly distributed in the cytoplasm (Figs. 1, 5).

Type IV: These are monoaxonal, more or less oval, each containing a large vacuole and an eccentric nucleus with 2 nucleoli (Figs. 1, 6). The fine NSG mainly aggregate near the cell periphery and in the axon.

Type V: These are large, irregularly-shaped cells, each containing 2 large vacuoles and a large central nucleus with one large nucleolus (Figs. 2, 7). The fine floculant NSG aggregate mostly at 2 sides of the nucleus.

Type VI: These are small, oval cells, with eccentric, spherical nuclei. Three subtypes are distinguished according to NSG distribution and or cell size. In subtype VIa, the NSG are uniform and evenly distributed (Figs. 2, 8). In subtype VIb some of the fine NSG accumulate around the nucleus (Fig. 2). In subtype VIc large aggregates forming droplets are scattered among the fine granules (Fig. 2).

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Type VII: These are the smallest (P < 0.001) NSC. They are rounded with relatively large nuclei and large NSG. Two subtypes VIIa and VIIb are observed according to NSG distribution; the granules are scattered evenly in the cytoplasm of the former and irregularly in the latter subtype (Fig. 2).

Type VIII: These are monoaxonal cells in which 2 subtypes are observed according to NSG distribution; the NSG are evenly scattered in subtype VIIIa (Figs. 2, 8) and accumulate in one side of the cell in subtype VIIIb (Fig. 2).

Type IX: In these oval cells, 2 subtypes are distinguished according to cell size and NSG distribution. The NSG are enclosed in areas of clear, unstained cytoplasm in the smaller (P < 0.01) subtype IXa cells (Figs. 2, 9) and are distributed more or less evenly in the cytoplasm of the larger subtype IXb cells (Figs. 2, 10).

Type X: These are oval, unipolar cells with a homogeneous cytoplasm which contains a peripherally located vacuole and a central nucleus with 2 small nucleoii. The fine to coarse NSG are irregularly distributed in the cytoplasm (Fig. 2).

Type XI: These are oval cells with relatively large, rounded central nuclei, each containing 1 or 2 large nucleoli (Fig. 3). The cytoplasm contains several small peripherally-located vacuoles. The fine NSG are distributed throughout the cytoplasm with heavy aggregates at one side of the nucleus.

Type XII: These are irregularly-oval cells with oval eccentric nuclei. Each containing 3 large nucleoli (Fig. 3). The fine NSG are scattered throughout the cytoplasm with colloidal droplets among them.

Type XIII: Two subtypes are observed in these irregularly-outlined cells according to their size and number of axons. The relatively smaller (P < 0.01) polyaxonal subtype XIIIa cells and the large monoaxonal subtype XIIIb cells possess a central nuclei, each with 2 nucleoli (Figs. 3, 11). The cytoplasm con-

tains several small vacuoles mainly at the cell periphery. The NSG are more heavily aggregated around the nucleus.

NSC distribution in the synganglion centers

Within the different cortical regions of the synganglion, the NSC are grouped in 13 centers. These groups are bilaterally symmetrical, each containing one or more cell types. Figure 12 and Table 2 illustrate the distribution of 22 neurosecretory groups in these centers.

Protocerebral center. This center includes 4 pairs of bilaterally symmetrical groups. Group 1, is the dorsal optic which consists of 2 or 3 type VIIa and 2 or 3 type X cells. Group 2, is anteroventral and consists of 4-6 NSC. It includes 2 type IIb cells, 1 or 2 type VIc cells and one type X cell. Group 3, is posterodorsal and consists of one type VIIb and 2 type XII cells. Group 4, is posterolateral and consists of 4 cells type IV, IX, X and XII and 2 type VIIb cells.

Cheliceral center. This center contains 3 pairs of bilaterally symmetrical groups. Group 5, is anterodorsal and consists of 2 type VIIa cells and one type IXa cell, Group 6, is ventral and consists of only one type IXa cell, while Group 7, is lateral and consists of 4 NSC of types VIa, VIIIa, VIIIa and IXb.

Stomodeal pons center. Two pairs of bilaterally symmetrical groups occur in this center. Group 8, is dorsal and consists of 2 type VIIa cells, and Group 9, is ventromedial and consists of 3 type VIIa cells.

Palpal center. This center contains 2 symmetrical groups. Group 10, is dorsolateral and consists of one type IIIa cell, and 3 or 4 type VIIIb cells, and Group 11, is ventrolateral and consists of 2 type V cells and one type VIIa cell.

Oesophageal center. This center includes the bilaterally symmetrical Group 12, which lies ventrolateral to the oesophagus and consists of 1 or 2 type VIIa, 2 or 3 type VIIb, and 2 or 3 type XI cells.

Table 1: Properties of the different neurosecretory cell (NSC) types in unfed female Hyalomma dromedarii synganglion.

	Cell	Mean cell dimensions (um)±SE	NSG.	PF.	NSC Staining reactions CHP**	reactions MT**
	I IIa	$55.0\pm0.03x23.5\pm0.62$ $30.8\pm0.02x22.0\pm0.03$	Very fine granules	Dark purple	Pale blue	Blue
	E E	$27.5 \pm 0.02 \times 20.9 \pm 0.03$ $44.0 \pm 0.03 \times 30.0 \pm 0.04$	Coarse granules	Bluish purple Purple to	Blue Pale Mine	Reddish blue Reddish blue
_	IIIb	35.0±0.03x25.0±0.03	granules Clumps of floc-	dark purple Purple to	rate blue Pale blue	Blue
47 —	7.	29.0±0.03x15.5±0.02	culent granules Fine granules	dark purple Orange to	Blue	Pale blue
-	>	$42.5\pm0.03x32.5\pm0.03$	Fine flocculent	purple Orange	Blue	Pale blue
	VIa,b	15.0 ± 0.04 x 11.0 ± 0.03	granuics Fine granules	purple Purple to reddish	Blue	Reddish blue
	VIc	18.0 ± 0.03 x 15.0 ± 0.04	Fine granules with large droplets	orange Purple to reddish	Blue	Reddish blue
	VIIa	12.5±0.04×11.5±0.02	among them Large granules	orange Purple to	Blue to	Blue to
	VIII	11.5±0.02x10.0±0.02	Large granules	dark purple Purple to	dark blue Blue to	dark blue Blue to
				arte barbie	Cark Dive	Ourk blue

(Cont. Table 1)

Cellitype	Mean cell dimensions (um) ±SE	• Day	- .	NSC Staining reactions CHP**	reactions MT • •
VIIIa	20.0±:0.02x 8.0± 0.20	Coarse granules	Dark reddish	Blue	Pale blue
VIIIb	20.0±0.02 x15 .0±0.03	Coarse granules	purple Dark reddish	Blue	Pale blue
IXa	15.0 · 0.03x 7.5 · 0.20	Coarse granules	purple Purple to	Blue	Reddish blue
. qX	24.2 - 0 02x 8.8 - 0.20	Coarse granules	blue Purple to	1,: Blue	Reddish blue
~ ×	17.5 - 0.03×10.0 : 0.92	Fine to coarse	blue Orange	r Pale blue	Reddish blue
· X	27.5 : 0.03x22.5 : 0.03	granules Fine granules with heavy	purple Dark purple	Pale blue	Blue to dark blue
пх	22.5 · 0.03x10.0 · 0 02	aggregates Fine granules with colloidal	Purple or orange	Blue	Blue or red
·	33 0±0.03×26.5±0 03	droplets among them	Bluish purple	Pale blue	Reddish blue
XIIID		granules Fine to medium	Bluish purple	Pale blue	Reddish blue

•NSG. = Neurosseretory granules ••PF = Ewen's paraldehyde-fuchsin, MT = Mallory Triple stain.

CHP = Gomori's chrome hematoxylin phloxine,

Table 2: Distribution of the neurosecretory cells (NSC) in the unfed female Hyalomma dromedaris synganglion.

Center and group No.	o. Location		NSC No. in each group	NSC type and location
Protocerebral center	١.			1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Group 1	Dorsal optic	(baired)	ĵ	2-3 X dornolateral
Group 2	Anteroventral	(paired)	4-6	2 IIb, 1-2 VIc, 1 X,
				anterodrosal to oesophages at its entry into
Group 3	Posterodorsal	(paired)	ო	synganglion 1 VIIb, 2 cells XII,
.		•		dorsal to posterodorsal glomeruli
Group 4	Posterolateral	(paired)	9	1 IV, 2 VIIb, 1 IXa, 1 X,
				i XII, doraolaterai to anterodorsal glomeruli
Cheliceral center				
Group 5	Anterodorsal	(paired)	es	2 VIIa, 1 IXa, near
· ·			٠	neurilemma
Group 6	Ventral	(paired)	Ħ	IXa, near posterior margin
Group 7	Lateral	(paired)	4	1 VIa, 1 VIIa, 1 VIIIa,
•		•		1 IXb. near neurilemma

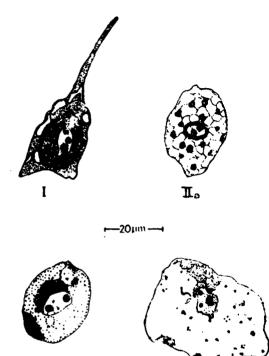
(Cont. Table 2)

Center and group No.	Location		NSC No. in	NSC type and location
Stomodeal Pons center	ter			
Group 8	Dorsel	(paired)	73	VIIA
Group 9	Ventromedial	(paired)	က	VIIs, surrounding oeso- phagus at its entry to synganglion
Palpal center				
Group 10	Dorsolateral	(paired)	2	1 IIIa, 3-4 VIIIb
Group 11	Ventrolateral	(paired)	ო	2 V, 1 VIIa, noar anterior margin of first pedal ganglia
Oesophageal center				
Group 12	Ventrolateral	(paired)	2-9	1-2 VIIIa, 2-3 VIIb, 2-3 XI, after oesophagus enters the synganghion
Globular center				
Group 13	Anteroventral	(paired)	2 3	1 VIIa, 1-2 VIIb
Offsetory glomerular center Group 14 Ventra	r center Ventra	(paired)	10	2 Id. 2 VID, 2 XIID

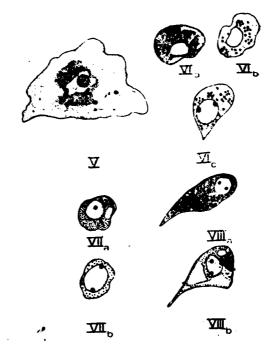
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(Cont. Table 2)

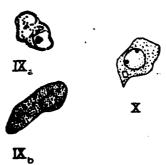
Center and group No.	Location		NSC No. in each group	NSC Type and Location
Pedal center				
Group 15	Dorsolateral	(paired)	4	1 IIIa, 1 IIIb near
		ı		neurilemma, 1 VIb deeper in cortex, (1 VIIa, 1 VIIb) adiacent to neuronile
Group 16	Ventrolateral	(paired)	4	2 VIIa adjacent to antero
				median surface of following ganglion, 2 VIIb near neurilemma
Opisthosomal center				
Group 17	Dorsomedial	(unpaired)	5-7	2 IIb, 1 IV, 3 VIIa,b,
				1 VIIIb
Group 18	Dorsclateral	(paired)	7	1 IIIb, 1 XIIIa
Group 19	Ventromedial	(unpaired)	79	1-2 I, 5 VIIa, 1 XII,
				1 XIIIb
Group 20	Ventrolateral	(paired)	2—9	3-4 VIIa, 3 VIIIb
Group 21	Posterior	(unpaired)	6	3 IIa, 2 VIIa, 2 VIIb,
				1 XIIIa, 2 XIIIb
Postoesophageal center	er			
Group 22	Postoesophageal	(paired)	8 -10	4 IIb, 1 IIIa, 3-5 VIIa,
				posterior to oesophagus
				exit from syngangion



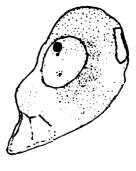
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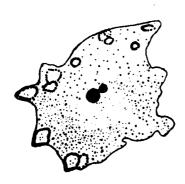
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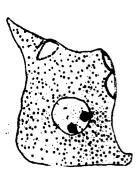




XI -

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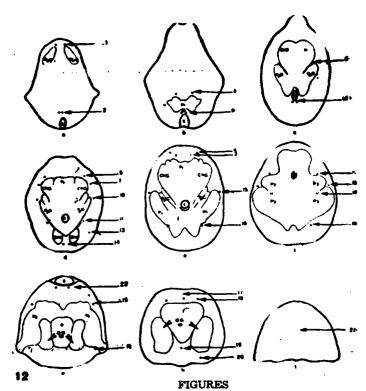


3.

XII_a

XIII

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Figs. 1-3: Diagrammatic illustrations of the neurosecretory cell types I-XIII in unfed female *Hyalomma dromodarii*.

Figs. 4-11: Transverse sections in the synganglion of unfed female *Hyalomma dromedarii* showing different neurosecretory cell types (arrows). X1500.

4. Type IIb. 5. Type IIIb. 6. Type IV. 7. Type V. 8. Types VIIIa (a) and VIa (b). 9. Type IXa. 10. Type IXb. 11. Type XIIIa.

Fig. 12: Diagrammatic illustrations (a-i) showing the distribution of the neurosecretory groups (1-22) in the different ganglia of unfed female Hydlomma dromedarii synganglion. ChG, cheliceral ganglia; E, oeophagus; OG, opisthosomatic ganglion; OfK, olfactory knots; OpG, optic ganglia; P 1-4, first to fourth pedal ganglia; Pp G, pedipalpal ganglia; protoeerebrum; St, stomodeal pons.

Globular center. This center contains one pair of hilaterally symmetrical Group 13, which is anteroventral to the globular cells and consists of 1 type VIIs and 1 or 2 type VIIb cells.

Olfactory glomerular center. This center contains one pair of bilaterally symmetrical Group 14. This is ventral and consists of 2 type IIb, 2 type VIIb, and 2 type XIIIb cells.

Pedal centers. In each of the 1st, 2nd, 3rd and 4th pedal ganglia occur'2 pairs of bilaterally symmetrical groups (15 and 16) with a similar pattern of NSC distribution. Group 15, lies dorsolateral in each pedal ganglion and consists of 5 cells, types IIIa, IIIb, VIb, VIIa and VIIb. Group 16, lies ventrolateral in each pedal ganglion and consists of 2 type VIIa cells and 2 type VIIb cells.

Opisthosomal center. This center contains 3 unpaired and 2 paired, bilaterally symmetrical groups. The unpaired Group 17 is dorsommedial and consists of 5-7 NSC, 2 type IIb, one type IV, 1 type VIIa, 2 type VIIb, and one type VIIIb cells. The paired Group 18, is dorsolateral and consists of one type IIIh and one type XIIIa cell. Group 19, is unpaired and ventromedial; it consists of 7-9 cells, 1 or 2 type I, 5 type VIIa, one type XII cells and one type XIIIb. Group 20 is paired and ventrolateral; it consists of 6-7 NSC; 3 or 4 cells type VIIa and 3 type VIIIb. Group 21, is unpaired and posterior; it consists of 3 type IIa, 2 type VIIa, 2 type VIIb, one type XIIIa and 2 type XIIIb cells.

Postoesophageal center. This center contains a pair of bilaterally symmetrical Group 22, which consists of 4 type IIb, one type IIIa and 3-5 type VIIa cells.

DISCUSSION

Thirteen different NSC types have been observed in the unfed female H. dromedarii synganglion according to the cell shape, size and staining reaction of their contents. Quantitatively, these types are similar to those described in the ixodid Dermacentor variabilis (Obenchain, 1974b) and the argasid

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Ornithodoros tholosani (Gabbay and Warburg, 1977). Only 2 types, A and B, were found in O. moubata (Eichenberger, 1970) one type in A. persicus (Eisen et al., 1973) and 2 types a and b in R. sanguineus (Chow and Wang, 1974).

The form, quantity and or distribution of the NSG in most of the different NSC types in H. dromedarii differ from those observed in other ixodid ticks (Obenchain, 1974b). Nevertheless, the NSG chromophilia in certain cell types in H. dromedarii is similar to that observed in certain NSC types of D. variabilis (Obenchain, 1974b) and O. tholozani (Gabbay and Warburg, 1977), yet most NSC in H. dromedarii are larger than those described in D. variabilis. Types I, II, VII and XI among H. dromedarii NSC contain NSG which stain purple to dark purple with PF, and are thus, similar to types I, VII, IX and X in D. variabilis (Obenchain, 1974b) and type II in O. tholozani (Gabbay and Warburg, 1977). This chromophilia indicates that the secretory material contains high levels of cysteine (Obenchain, 1974b).

Comparison between PF staining reactions of NSG in cell types of both H. dromedarii and D. variabilis (Obenchain, 1974b) indicates that types VI in the former stains purple to reddish orange as type V in the latter, while type VII in the former resembles types II and XI in the latter; they all stain dark reddish purple. Types II, IX, and XIII in H. dromedarii stain bluish purple as types III, IV and VI in D. variabilis and types I and V in O. tholozani (Gabbay and Warburg, 1977). NSG staining reddish or bluish purple with PF seem to have a higher carbonyl content than those which stain dark purple (Obenchain, 1974b).

Types IV, V, X, XII in *H. dromedarii* contain an acidophilic secretory product with a staining affinity to the orange G in PF technique, thus simultating type XIIIa in *D. variabilis* and type X in *O. tholosomi* (Obenchain, 1974b; Gabbay and Warburg, 1977). Acidophilia persisting after oixdation may be attributed to proteins rich in tryptophan (Raabe and Monjo, 1970; Baudry and Baehr, 1970). Cytoplasmic affinity for the light

green counterstain of CHP technique does not exist in *H. dro-medarii*, although present in *D. variabilis* (Obenchain, 1974b) and *O. tholozani* (Gabbay and Warburg, 1977).

Grouping of NSC varies in ixodids, being fifteen groups in B. microplus (Binnington and Tatcheil, 1973), eighteen groups in D. pictus (Ioffe, 1964), eighteen centers in D. variabilis (Obenchain and Oliver, 1975), and eighteen centers in I. persulcatus (Panfilova, 1981). Dhanda (1967) recognized eighteen NSC centers in H. dromedarii synganglion, without specifying their locations, but here we observed only thirteen centers containing 22 groups in this tick.

The fifteen and eighteen groups of NSC observed in B. microplus and D. pictus, respectively (Binnington and Tatchell, 1973; Ioffe, 1964) are recognizable within the thirteen NSC centers here described in H. dromedarii as well as the eighteen centers in D. variabilis (Obenchain and Oliver, 1975). Differences in the centers number in the two latter ixodids is mainly due to the inconsistent classification of NSC in the cheliceral, pedipalpal, and opisthosomal ganglia and stomodeal pons, into more than one center. In D. variabilis (Obenchain, 1974b) cach neurosecretory component contains single typed-cells whereas in H. dromedarii it contains one or more cell types. As suggested by Obenchain (1974a) the small numbers of cells in each neurosecretory group seems to reflect an inherent economy which is also expressed in the condensation and miniaturization of acarine central nervous system.

In the present study, NSC presently demonstrated in the unfed female *H. dromedarii* require further study to elucidate their activity in hormonal secretions during and after feeding and their role in the regulation and coordination in the physiological events occurring during these phases. Studies on these parameters are underway.

ACKNOWLEDGEMENTS

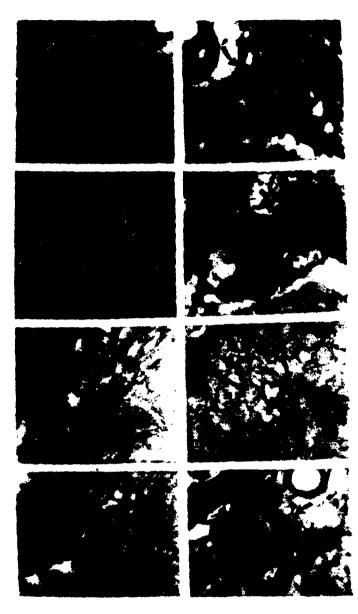
We are greatly indebted to Professor Mohamed A. Roshdy,

Department of Zoology, Ain Shams University and Scientific Consultant at Medical Zoology Department, NAMRU-3, Cairo, for his encouragement and avluable comments, and for critically reading the manuscript. Thanks are also due to Dr. William H. Dees, Head, Medical Zoology Department, for his interest in this work.

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CURITY	CLASSIFIC	ATION	OF	THIS	PAGE

REPORT DOCUMENTATION PAGE							
1a. REPORT SECURITY CLASSIFICATION	*****	16 RESTRICTIVE	MARKINGS				
UNCLASSIFIED 24. SECURITY CLASSIFICATION AUTHORITY		3 DISTRIBUTION	AVAILABILITY OF	REPORT			
		•	for public				
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE		Distribut	ion is unli	mited			
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING C	RGANIZATION RE	PORT NUMBER	5)		
19/88							
U.S. Naval Medical Research	O OFFICE SYMBOL (If applicable) AVMEDRSCHU HREE	7a NAME OF MO	INITORING ORGAI	NIZATION			
6c. ADDRESS (City, State, and ZIP Code)	HREE	7b. ADDRESS (Cit)	y, State, and ZIP (ode)			
PPO New York 09527-1600 Re NAME OF FUNDING / SPONSORING RED OFFICE SYMBOL 9 PROCUPEMENT INSTRUMENT IDENTIFICATION NUMBER							
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command NAVMEDRSCHDEV COM							
8c. ADDRESS (City, State, and ZIP Code) 10. SOURCE OF FUNDING NUMBERS							
Naval Medical Command, National Capital Region PROGRAM PROJECT TASK WORK UNIT							
Bethesda, MD 20814-5044 ELEMENT NO. NO.3M1611- NO ACCESSION NO 61102A 02BS10 AD DA301560							
11. TITLE (Include Security Classification)							
Neurosecretory Cell Types and Distribution in Unfed Female <u>Hyalomma</u> <u>dromedarii</u> (Acari: Ixodoidea: Ixodidae) Synganglion. (UNCLASSIFIED)							
12 PERSONAL AUTHOR(S) Marzouk, A.S.*, Khalil, G.M.**, and Darwish, Z.E.A.+							
Marzouk, A.S.*, Khalil, G.M.**, and Darwish, Z.E.A.+ 13a TYPE OF REPORT 13b TIME COVERED 14 DATE OF REPORT (Year, Month, Day) 15 PAGE COUNT							
FROMTO1987							
16 SUPPLEMENTARY NOTATION Published in: J. Egypt. Soc. Parasitol., 17(1):41-61, 1987; Acc. No. 1500.							
17 COSATI CODES 1	8 SUBJECT TERMS (C	Continue on reverse	if necessary and	identify by bloc	k number)		
FIELD GROUP SUB-GROUP	Ticks; Hyalo	omma dromedar	ii; Neurose	cretory cel	.1;		
	Distribution	r; Types.					
19. ABSTRACT (Continue on reverse if necessary and	d identify by block n	umber)					
Thirteen NSC types have been obse	rved in the un	nfød female F	iyalomma dro	medarii syr	ganglion.		
Distribution of these types was b	ased on cell s	shape, size a	nd staining	reaction o	of their		
contents. The NSC are grouped in							
pons, palpal, oesophageal, globul				opistnosoma	il and		
post-oesophageal. Each center contains one or more cell types.							
* Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.							
** Present address: Zoology Depar	-				ar.		
+ Zoology Department, Faculty of							
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20 DISTRIBUTION/AVAILABILITY OF ABSTRACT 24UNCLASSIFIED/UNLIMITED 3 SAME AS RPT.	DTIC USERS	21. ABSTRACT SEC UNCLASSII	CURITY CLASSIFICA PIED	ATION			
22a NAME OF RESPONSIBLE INDIVIDUAL	SEA OTHE OSERS	226 TELEPHONE	nclude Area Code		/MBOL		
Research Publications Branch DD FORM 1473.84 MAR 83 APR e	dition may be used un	20-2-820-		R.P.B.			

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